

## Article

# Histo-blood group antigen-producing bacteria cocktail reduces rotavirus A, B and C infection and disease in gnotobiotic pig-lets

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## SUPPLEMENTARY MATERIALS

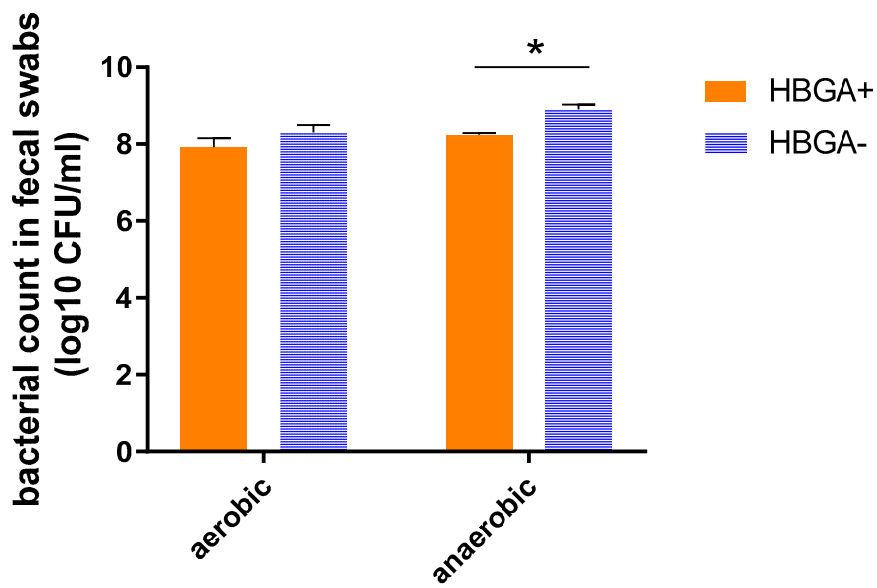
**Table S1.** Primers used in this study.

Target Organism	Primer	Sequence (5'-3')	Reference
<i>S. bovis</i>	F	ATGTTAGATGCTTGAAAGGAGCAA	(43)
	R	CGCCTTGGTGAGCCGTTA	
<i>B. thetaiotaomicron</i>	F	CGTTCCATTAGGCAGTTGGT	(44)
	R	CGTAGGAGTTTGGACCGTGT	
<i>C. clostridiiforme</i>	F	AATCTTGATTGACTGAGTGGCGGAC	(45)
	R	CCATCTCACACTACCGGAGTTTTTC	
<i>B. adolescentis</i>	F	CTCCGCCGCTGATCCGGAAGTCG	(46)
	R	AACCAACTCGGCGATGTGGACGACA	
<i>B. longum</i>	F	CGGCGTYGTGACCGTTGAAGAC	
	R	TGYTTCGCCRTCGACGTCCTCA	
<i>L. brevis</i>	F	CTTCTGGATGATCCCGCGGCG	(47)
	R	ACCGCCTGCGCTCGCTTTAC	
<i>E. coli</i> G58	F	GCGAGGTAACCTCGAACATG	(48)
	R	CGCCGTATCGATAATTCACG	
Rotavirus B	F	GGTTTAAATAGCCCAACCGGTGC	(52)
	R	TGCAATTTRATGCATGCGTT	
	Probe	FAM/AGCATGGATCTGATYGAAACRG	

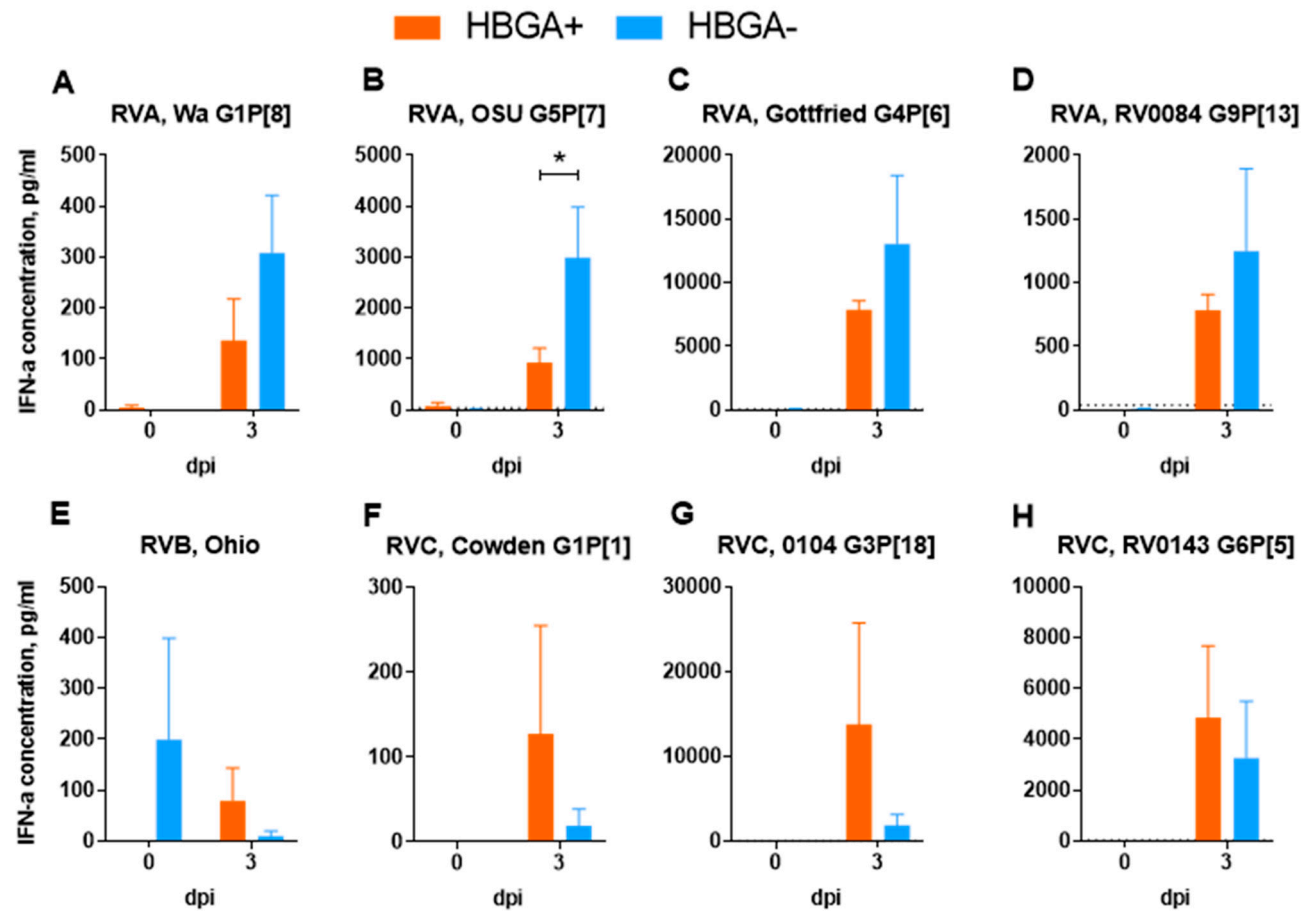
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Rotavirus C	<b>F</b>	ATGTAGCATGATTACGAATGGG	(51)
	<b>R</b>	ACATTTCATCCTCCTGGGGATC	
	<b>Probe</b>	FAM/TTGCGTAGGGGCAAATGC	

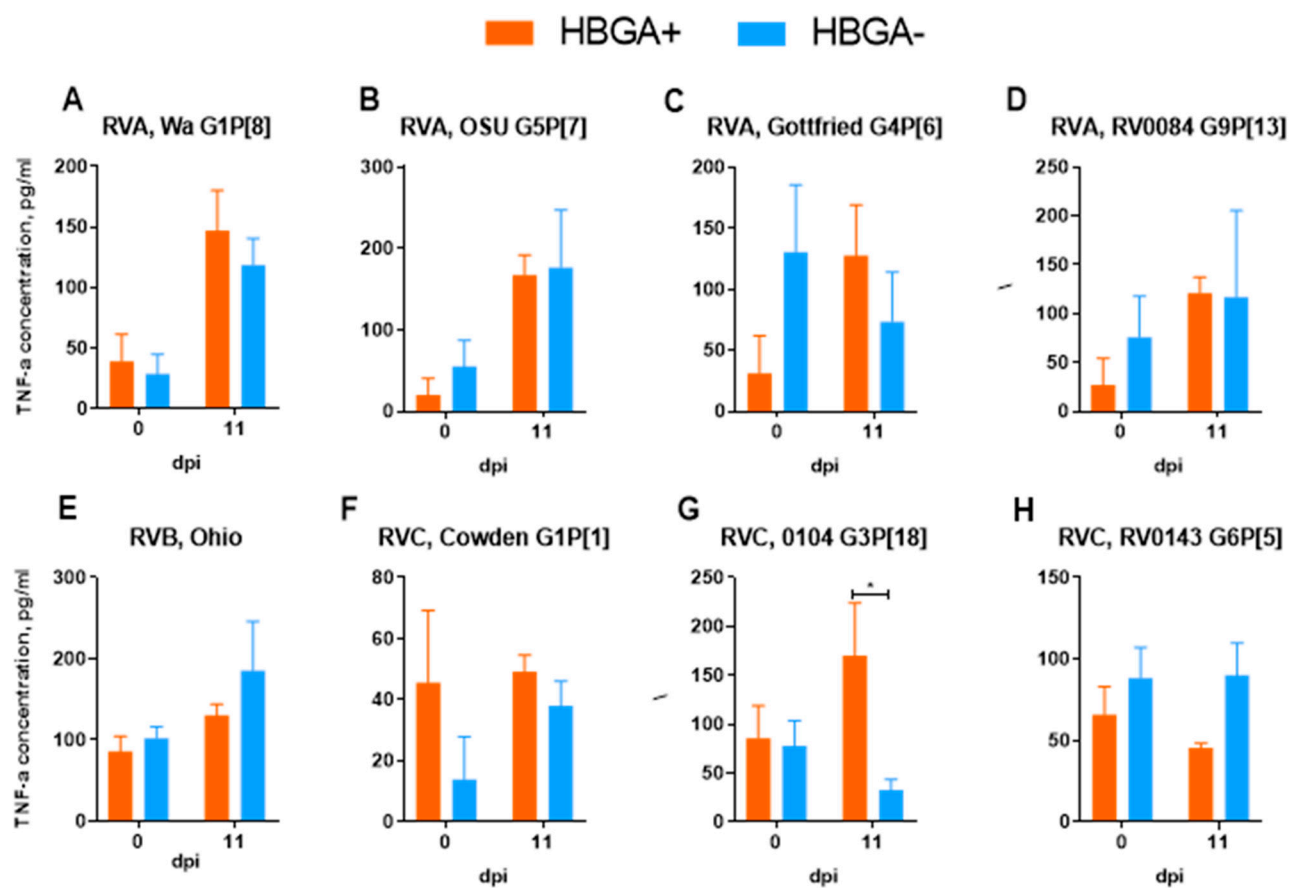
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**Figure S1.** Fecal probiotic bacterial shedding from probiotic colonized piglets. An asterisk indicates significant difference ( $p < 0.05$ ) in fecal probiotics counts among treatment groups.



**Figure S2.** IFN- $\alpha$  concentrations in piglet blood samples collected on dpi 0 and dpi 3 following RVA (A-D); RVB (E) and RVC (F-H) infection. Significant differences (\*  $p < 0.05$ ) are indicated as calculated by two-way ANOVA followed by Sidak's multiple comparisons test.



**Figure S3.** TNF- $\alpha$  concentrations in piglet blood samples collected on dpi0 and 11 following RVA (A-D); RVB (E) and RVC (F-H) infection. Significant differences ( $* p < 0.05$ ) are indicated as calculated by two-way ANOVA followed by Duncan's multiple comparisons test.

Table S2. Diarrhea and virus shedding in gnotobiotic piglets after oral inoculation with virulent RVs.

RV specie s	HBGA phenotype	Diarrhea										Viral shedding							
		N		Mean days to diarrhea onset <sup>1</sup>		Mean diarrhea duration (days) <sup>2</sup>		Mean cumulative fecal score <sup>3</sup>		AUC <sup>4</sup>		Mean days to shedding onset		Mean shedding duration (days)		Avg peak titer (FFU/ml)		AUC <sup>4</sup>	
		Bacterial cocktail																	
		HBGA <sup>+</sup>	HBGA <sup>-</sup>	HBGA <sup>+</sup>	HBGA <sup>-</sup>	HBGA <sup>+</sup>	HBGA <sup>-</sup>	HBGA <sup>+</sup>	HBGA <sup>-</sup>	HBGA <sup>+</sup>	HBGA <sup>-</sup>	HBGA <sup>+</sup>	HBGA <sup>-</sup>	HBGA <sup>+</sup>	HBGA <sup>-</sup>	HBGA <sup>+</sup>	HBGA <sup>-</sup>	HBGA <sup>+</sup>	HBGA <sup>-</sup>
RVA	H <sup>+</sup> A <sup>-</sup>	3	2	1.7	1.5	3.0	6.5	12	18.5	11	17.7	2	1.5	6	7.5	7.50E+04	1.49E+07	2.41E+06	2.58E+07
	H <sup>+</sup> /H <sup>-</sup> A <sup>+</sup>	12	13	2.3	2	3.7	4.8	12.3	14.5	11.8	14	1.8	1.7	6.3	7.2	4.69E+06	3.30E+06	9.47E+06	6.95E+06
RVB	H <sup>+</sup> /H <sup>-</sup> A <sup>+</sup>	4	3	2	1	5.2	7.7	13.5	19.7	13.5	19.7	1	1	7	7	1.05E+03	1.88E+03	2.07E+03	2.44E+03
RVC	H <sup>+</sup> A <sup>-</sup>	5	4	3.8	2.9	4.2	5	14.4	15.5	13.9	14.7	2.4	1.7	8.6	10	2.00E+06	2.00E+05	3.13E+06	3.13E+06
	H <sup>+</sup> /H <sup>-</sup> A <sup>+</sup>	6	11	2.7	2.5	4.2	5.1	13.5	15.5	13	14.9	2	1.4	9.2	10	1.54E+06	7.39E+05	1.71E+06	1.71E+06

<sup>1</sup>Diarrhea onset is defined as the number of days between the virus inoculation and the first manifestation of diarrhea with a score not less than 2. <sup>2</sup>Duration of diarrhea is defined as the number of days that the diarrhea score was 2. Fecal diarrhea was scored as follows: 0, normal; 1, pasty; 2, semiliquid; 3, liquid. <sup>3</sup>Mean cumulative fecal score [(sum of fecal consistency score for 11 days postinoculation)/N], where N is the number of pigs receiving the inoculation. Means in the same row were analyzed by unpaired t-test. <sup>4</sup>Area under the curve (AUC) was calculated using the area under the curve analysis function in the Prism software. A Kruskal-Wallis rank sum test was then performed to compare the total AUC between the groups. Significant differences (**bold**) are indicated as calculated by unpaired t-test.

**Table S3.** Data on the ability of individual HBGA+ and HBGA- bacterial strains to bind virulent RVs (16).

HBGA expression status	Strain	RV-bacteria binding				
		RVA				RVC
		G5P[7]	G9P[13]	G4P[6]	G1P[8]	G1P[1]
HBGA+	<i>S. bovis</i>	no	no	no	no	no
	<i>B. thetaiotaomicron</i>	no	no	no	yes*	no
	<i>C. clostridiiforme</i>	yes***	yes***	no	no	no
	<i>B. adolescentis</i>	no	no	yes*	yes*	yes*
	<i>E. coli G58</i>	no	yes**	yes***	yes**	no
HBGA-	<i>B. longum</i>	no	no	no	no	no
	<i>L. brevis</i>	no	no	no	no	no

The table represents the difference in the amount of bacteria-bound RV. \* P<0.05, \*\* P≤0.01, \*\*\* P≤0.001.